

THE PHYSIOLOGY OF THE DEVELOPMENT OF
THE IMAGINAL BUDS IN THE BAR SERIES
OF *DROSOPHILA MELANOGASTER*

BY

CHARLES RALPH WILLIAMS

A. B., University of Illinois, 1937

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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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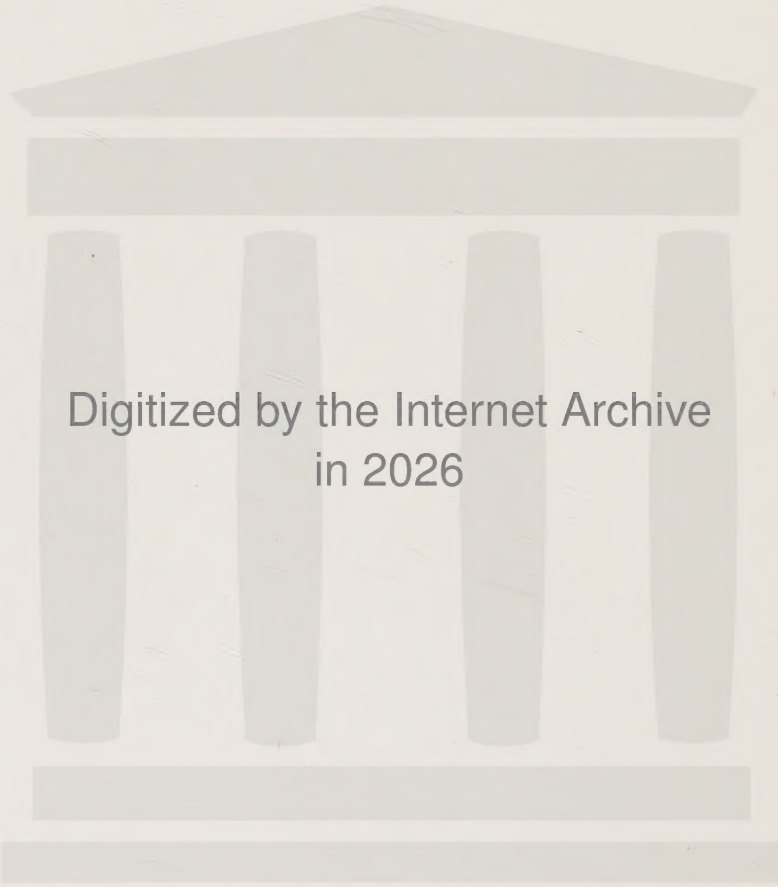
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PROLONGATION OF LARVAL-PUPAL DEVELOPMENT IN *DROSOPHILA MELANOGASTER* AND ITS EFFECT ON FACET NUMBER¹

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INTRODUCTION

THERE are several instances in the literature in which the effect of prolongation of developmental time and the resultant effect on gene expression have been reported. Only a few of these reports were concerned with facet number in *Drosophila melanogaster*. Braun (1939) and Green and Oliver (1943) investigated the effect of prolongation of development in the larval stage on the expression of genes for wing characters in *Drosophila melanogaster*. They postulated the elaboration of a substance by the factors involved. Prolongation of development enhanced the expression of the responsible factors by allowing additional time for elaboration of the substances which resorb the already fully formed material that would normally form the character as in the case of notched wing as studied by Braun. Green and Oliver, in brief, report on experiments with vestigial wing in which they found that prolongation of developmental processes resulted in an increased expression of the vestigial character due to an increase in the amount of substance elaborated by the factor responsible. Luce (1939, 1940, 1942) studied the effects of formalin, of oxygen and of a mixture of oxygen and carbon dioxide on facet number in bar alleles of *Drosophila melanogaster*. He found (1942) that high concentrations of formalin increased the length of development and reduced the mean facet number. In this instance the lengthening of development enhanced the expression of the bar factors. The oxygen studies of

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the same author conducted on a bar infrabar strain (1940) produced different results. The time of development was prolonged, but the expression of the gene difference at the bar locus was decreased as the facet number of the flies was greater than in the controls. His studies on the effect of a mixture of 95 per cent. oxygen and 5 per cent. carbon dioxide (1939) yielded similar results in an infrabar strain.

VITAMIN B₁ (THIAMIN HYDROCHLORIDE)

The present study was carried out upon bar and ultra-bar strains of *Drosophila melanogaster* and dietary deficiencies were utilized to induce prolongation of development. Schader (1941) noted a prolongation of larval development due to a diet deficient in vitamin B₁. Keenan, Kline, Elvehjem and Hart (1935) devised a technique for inactivating vitamin B₁ in fresh cake yeast. Van't Hoog (1935) formulated a synthetic culture medium for insects which could be prepared and kept sterile. Baumberger (1919) found that microorganisms were a source of nutrition to insects. He also showed that autoclaved yeast was a completely adequate food for *Drosophila melanogaster*.

VITAMIN E

There is no conclusive evidence that insects need any other vitamin than vitamin B for normal development. Hill and Burdett (1932) believed, however, that the royal jelly of honey bees, which is the substance necessary for the transformation of worker larva to the queen or sexually mature form, was a rich source of vitamin E. They interpreted their results as indicating the presence of appreciable amounts of the vitamin in royal jelly, but subsequent investigations by Mason and Melampy (1936), Evans, Emerson and Ackert (1937), and Haydak and Palmer (1939) failed, after careful assay, to reveal any vitamin E in this substance. The utilization of vitamin E inactivation methods (Waddell and Steenbock, 1928) were

employed on the culture medium used in part of this study to determine if this vitamin is essential in *Drosophila* growth and development.

MATERIAL AND METHODS

The strains of flies, bar and ultrabar, used in this experiment had a sufficiently reduced number of facets so that the "error factor" in counting facets was greatly minimized. These strains were originally derived from a bar-eyed line inbred since 1923, at a constant temperature of 27° C. This is the so-called "temperature-inheritance line" of the University of Illinois.

The culture medium used for the stock flies from which individuals were obtained for the diverse experiments is a modification of the Russian formula by Luce (unpublished). It comprises these ingredients:

80	ml	biological water (chlorine free)
1.5	gm	agar agar
1.5	"	dried brewer's yeast
6.0	"	yellow corn meal
6.0	"	syrup
5.0	"	ground raisins
		a small amount of powdered yeast
		foam to each vial

The water, agar agar and corn meal were heated and allowed to boil until the ingredients were almost cooked. The brewer's yeast, syrup and raisins were then added, and all were permitted to boil for several minutes. The food was then placed in 25 × 100 mm shell vials, about one inch of food to each vial. Powdered yeast foam was sprinkled lightly on the food surface after it had cooled and solidified.

VITAMIN E DEFICIENCY

The vitamin E inactivated medium was obtained by treating the corn meal and raisins of the medium with ferric chloride and ether (Waddell and Steenbock, 1935). One per cent. by weight of ferric chloride, which had been powdered, mixed with a quantity of ether equal to the volume of the food treated was the mixture used for in-

activation of the vitamin E contained in the food. The oxidizing medium and the food treated were kept in a closed vessel for 24 hours to permit extraction of the fat soluble vitamin and the oxidation of it. After 24 hours, the ether was allowed to evaporate by removing the cover of the vessel. After complete evaporation of the ether, the treated food was ready for use in the preparation of the experimental medium. To check on the possibility of lethal effects or toxicity due to the presence of the ferric chloride, a second medium was prepared which contained an amount of ferric chloride equal to that used in the inactivation process.

VITAMIN B₁ DEFICIENCY

A vitamin B₁ deficient medium was prepared synthetically (Van't Hoog, 1935). It contained agar agar, sucrose, yeast and Pearl's salt mixture, modified by Sang. The salt mixture consisted of three different solutions:

SOLUTION A

8.00 gm	potassium sodium tartrate
2.00 "	ammonium sulfate
0.50 "	magnesium sulfate
250.00 ml	distilled water

SOLUTION B

5.00 gm	tartaric acid
0.65 "	potassium dihydrogen phosphate
250.00 ml	distilled water

SOLUTION C

0.25 gm	calcium chloride
250.00 ml	distilled water

The solutions were autoclaved for 20 minutes at 250° F. and under 15 pounds of steam pressure. In the preparation of the experimental medium, 10 grams of Fleischmann's fresh cake yeast was added to a flask containing 25 ml of distilled water. This was autoclaved for 5 hours at 250° F. and under 15 pounds of steam pressure. This treatment inactivated the thiamin hydrochloride present in the yeast (Keenan, Kline, Elvehjem and Hart, 1935).

Schader (1941) and others have found that this inactivation process also affects the potency of riboflavin and nicotinic acid present in the yeast. Because of this it was necessary to add these vitamin B components in the proportion of 14.3 mg of nicotinic acid and 0.05 mg of riboflavin to each 100 ml of the culture medium. The autoclaved yeast was added to 25 ml of solution A and 25 ml of solution C. The nicotinic acid, riboflavin, 1.5 grams of agar agar and 1 gram of sucrose were added to this mixture. Twenty-five ml of solution B were contained in another flask. The two flasks were autoclaved for 20 minutes at 250° F. and under 15 pounds of steam pressure. Solution B was then mixed with the contents of the other flask and the resultant mixture was boiled for one minute. Three ml of this mixture were transferred to sterile cotton-stoppered, 25×100 mm shell vials by means of sterile pipettes. The food was allowed to cool and solidify. It was important that the steps as outlined be followed, since hydrolization of the agar agar occurred if this precaution was not observed. In preparing the control and check media, the same procedure was observed except that in the control the yeast was not autoclaved for more than twenty minutes. Also the riboflavin and nicotinic acid were not added. In a check medium experiment the culture medium consisted of the experimental medium plus an amount of thiamin considered to be equivalent to that inactivated. The thiamin added was contained in a sterile aqueous solution. The amount added was 0.08 mg/100 ml of culture medium.

PARENT FLIES AND EGG COLLECTING

The stocks of flies used in the study had been closely inbred at a constant temperature of 24° C. $\pm$ 0.5°. From these stocks pure male and female ultrabar and bar flies were selected. Eight males and 8 females were placed in each vial containing the stock culture medium. They were inbred for several more generations and then used for this study.

The method of collecting eggs is original and permits of securing large numbers in a short time. It also facilitates handling of eggs in transferring them to vials. Strips of absorbent paper-toweling were cut to fit into the shell vials. Onto these paper strips a 1 per cent. agar agar solution was dropped in the shape of oval buttons. After the agar had solidified, the "buttons" were grooved with a needle, painted with a solution of 2 parts of glacial acetic acid, 10 parts sorghum molasses and 88 parts distilled water. The paper strips were then transferred to the shell vials containing 8 pairs of the stock flies to be used for parents. The vials containing the strips of paper were placed in a lidded, metal chamber lined with moistened absorbent paper. Thus all the factors conducive to greater egg production were supplied. The ovipositing period was of four hours' duration and the age of the egg-larva could be determined with a - 2 hour error if the midpoint of the egg-laying period was used as the time of oviposition (Alpatov, 1929).

To collect the eggs the agar "buttons" were placed in a Petri dish after removal from the paper strips. They were observed under a binocular microscope and a determination of the number of eggs on each "button" was made. The eggs were rendered aseptic as follows. A sufficient volume of 85 per cent. alcohol was poured into the Petri dish to cover the "buttons." This treatment destroys any yeast cells which might be clinging to the eggs but does not injure the embryo within (Schader, 1941). According to Baumberger (1919), yeast cells are not transmitted from the adult female to the eggs. This procedure greatly obviated the possibility of contaminating the culture media used in these experiments. After one hour in 85 per cent. alcohol, the agar "buttons" were transferred to vials with a sterile dissecting needle.

TEMPERATURE CONTROL, ASEPSIS AND CALCULATIONS

The experiments of the present study were all carried out at the optimum temperature for *Drosophila melano-*

gaster development, *i.e.*, $24^{\circ}\text{C.} \pm 0.05^{\circ}$. This was accomplished by the use of a constant-temperature chamber specially constructed and well insulated containing both cooling and heating units under automatic control. A thermograph made constant recordings of the temperature.

Aseptic conditions were maintained throughout the study by autoclaving all cultural media, food vials, pipettes, etc. The vials used for flies were flamed whenever transfers were made, the eggs were placed in 85 per cent. alcohol for one hour, and all contaminated vials were immediately discarded.

Hourly observations were made during the critical developmental periods and a record of time spent in larval and pupal development was obtained. The facets of the compound eyes of random samples of flies obtained from these experiments were counted under an E. Leitz, Ultrapak microscope. The random sample consisted of 20 to 40 individuals of each sex for each culture medium used. Statistical constants were obtained from these samples.

EXPERIMENTAL RESULTS

The experimental results are presented in tabular form comparing length of developmental times, the mean of the facet number for males and females, standard deviation of the mean and standard error of the mean. Statistical significance is also indicated in these tables.

In all the experiments conducted the rate of mortality, the fertility and the sex ratio of the flies were essentially normal.

DISCUSSION

The data obtained from this study are significant in several respects. From a nutritional viewpoint it is evident that thiamin hydrochloride is not necessary to complete development though it is necessary for normal development. This would be in accord with the results of Lafon's findings (1935) and Schader's (1941): A study

of Table 1 would indicate that the amount of thiamin added to the culture medium which had been subjected to vitamin B₁ inactivation was not equivalent to the amount inactivated, for we find an extension of the developmental periods where this medium is employed as well as a significant increase in facet number. It can be concluded that this medium was partly deficient in thiamin, which

TABLE 1

PROLONGATION OF LARVAL AND PUPAL STAGES OF DEVELOPMENT DUE TO VITAMIN B₁ DEFICIENCY AND A CONSEQUENT INCREASE IN FACET NUMBER IN A BAR STRAIN

	I Control	II Experimental organisms cultured on B ₁ inactivated food to which .08 mg of B ₁ /100 ml of culture medium was added	III Experimental organisms cultured on a medium treated for inactivation of Vitamin B ₁
Mean larval period in hours	190	202	323
Mean pupal period in hours	110	120.5	167
Facet number			
Males	132.10	150.45	162.28
Females	83.10	87.31	95.00
Standard deviation of the mean			
Males	± 5.27	± 3.67	± 4.75
Females	± 6.32	± 2.92	± 4.55
Standard error of the mean			
Males	± .96	± .62	± .91
Females	± 1.38	± .61	± .91
Statistical significance			
Males	+ with II	+ with III	+ with I
Females	+ with II	+ with III	+ with I

(+ = statistical significance.)

resulted in a reduced effect on both lengthening of development and facet number as compared to complete inactivation of thiamin in the other experimental medium.

It is not presumed that the inactivation of vitamin E was the causative factor in prolonging development in the ultrabar strain studied. The report of Tatum (1939) and some unpublished work by the author indicates that no other vitamin than vitamin B is essential to *Drosophila* development. It is evident that the addition of ferrie

chloride to the culture medium had no significant toxic effect. It is concluded that the vitamin E inactivation process had an effect on the lipids present in the food treated. It is altogether probable that some fat-fraction, yet unidentified, was oxidized by the treatment. Since the chief concern of this study is the factors which influence gene expression, it does not matter which food component is actually affected by the inactivation process.

TABLE 2

PROLONGATION OF LARVAL AND PUPAL STAGES OF DEVELOPMENT DUE TO VITAMIN E INACTIVATION AND A CONSEQUENT INCREASE IN FACET NUMBER IN AN ULTRABAR STRAIN

	I Control	II Experimental organisms cultured on a medium con- taining 1 per cent. ferric chloride by weight	III Experimental organisms cultured on a medium treated for Vitamin E inactivation
Mean larval period in hours	167	178	272.50
Mean pupal period in hours	120	131	154.00
Facet number			
Males	29.27	29.88	31.97
Females	26.00	27.19	29.27
Standard deviation of the mean			
Males	± 4.20	± 2.58	± 2.76
Females	± 2.42	± 2.42	± 3.36
Standard error of the mean			
Males	± .66	± .40	± .46
Females	± .46	± .46	± .67
Statistical significance			
Males	- with II	+ with III	+ with I
Females	- with II	+ with III	+ with I

(+ = statistical significance, - = absence of significance.)

The absence of thiamin and the inactivation process of vitamin E had no appreciable effect on the mortality rate, fertility or sex ratios, but they did slow up the physiological and metabolic activities which led to normal development. This slowing up with a consequent prolongation of development had a peculiar effect on the expression of the factors responsible for facet number. The expression of these gene-differences at the bar locus was

decreased rather than enhanced. Braun (1939) and Green and Oliver (1943) postulated an enhancement of gene expression for the characters studied when the developmental time of the larval stage was prolonged. This enhancement of expression was reflected in a more pronounced effect on the end-characters than when development was normal.

The conclusions of Braun are based on the hypothesis that the notched-wing character he studied was due to a resorption by substances elaborated by the gene for the character. The longer the time of development, the longer the time the gene has to act and hence the greater the amount of resorptive substance elaborated. Green and Oliver suggest that from the data they have obtained it would seem that the gene for vestigial wing belongs in the category of antimorphic genes. This would mean that the gene for vestigial acts to prevent normal differentiation of wings. Prolongation of development permits an increase in the time of gene action so that in the case of heterozygous *vg* sufficient substance to cause scalloping is produced. The quantity of vestigial substance elaborated depends upon the dosage of *vg*, the allele present and the extent of prolongation of the third larval instar.

It is evident from the results of the present study that the gene differences at the bar locus do not express themselves in a manner comparable to the factors studied by the above-mentioned authors. In the case of eye development, a study of the embryology has shown that there is no evidence that resorption takes place at any time (Steinberg, 1941). Also, from the data obtained it is not possible to associate the mode of gene expression for facet number with that for vestigial. In the latter case the prolongation of development enhanced gene expression, while in the former case it was decreased. If the gene differences for facet number at the bar locus were enhanced in their expression, then a greater reduction in facet number would result, but in the present study we have found that the facet number was increased by the prolonged developmental time.

It can be postulated that the factors for facet number are slowed in their expression by prolongation of the larval period which results in an increased number of facets. This postulation is bolstered by the investigations of Luce (1939, 1940 and 1942) as well as by the results of numerous investigations on temperature effects on *Drosophila melanogaster*.

SUMMARY

A diet deficient in thiamin hydrochloride greatly prolongs the larva-pupa time of development with a consequent effect on facet number in a bar strain of *Drosophila melanogaster*.

A culture medium treated for inactivation of vitamin E by use of a mixture of ferric chloride and ether not only has the vitamin E inactivated but some other food component which is thought to be a fat-fraction. This treated medium considerably prolongs larval-pupal development with a consequent effect on facet number.

The facet number of flies cultured on both types of culture media was increased significantly. This would indicate a decrease of gene expression due to the prolongation of development caused by the dietary deficiencies.

From these data it can be concluded that the factor responsible for facet number in *Drosophila melanogaster* does not function in a manner comparable to the so-called antimorphic gene *vg* which was studied by Green and Oliver (1943) nor the gene for notched-wing which elaborates resorptive substances as postulated by Braun (1939).

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VITA

Charles Ralph Williams was born December 8, 1909, in Chicago, Illinois, son of Mary M. and Ralph James Williams.

He attended parochial school in Chicago and finished his primary schooling in 1923. He finished high school at Fournier Institute, Lemont, Illinois, in 1928.

In 1933-34 he attended St. Viator College. In 1935 he transferred to the University of Illinois and received his A.B. degree from that institution in January of 1937 and the Master of Arts from the same institution in June of that year. During the summer of 1937, he attended the Marine Biological Laboratory at Woods Hole, Massachusetts.

He attended Catholic University Theological Seminary from 1937 to 1941 and was ordained to the priesthood in May of 1941. While at Catholic University, he obtained courses in chemistry, physics, and geology besides the formal courses in Theology.

He returned to the University of Illinois in September of 1941 to pursue studies leading to the Ph.D. degree. In 1941-42 he served as an assistant in the Department of Zoology. During the summer of 1943, he became associated with the faculty of De Paul University in Chicago, Illinois. During that summer he taught courses in comparative anatomy and genetics. He was granted a leave of absence for the year 1943-44 to complete the requirements of the University of Illinois for the Ph.D. degree.

He is a member of Phi Sigma Society, the Genetics Society of America, and the American Association for the Advancement of Science and an associate member of Sigma Xi.

